

the amino-terminus to the virus surface and the carboxy-terminus inside of the viral particle. In vitro studies have shown that synthetic coronavirus envelope proteins oligomerize forming pentameric structures with ion channel activity [2].

We have evaluated the ion channel activity of several synthetic peptides derived from the transmembrane domain of SARS-CoV and HCoV-229E E proteins by using planar bilayer electrophysiology of peptide oligomers in DPhPC membranes. The conditions under which some of these peptides form ion channels and their properties at the single channel level were determined. The ion channel activity of a collection of SARS-CoV E protein transmembrane domain derived peptides with point mutations showed that specific residues, mapping at the internal side of the pore, abolished the conductivity. The conductance of the wild type SARS-CoV and HCoV-229E E protein derived peptides was studied under different salt concentrations. At 1M solutions of monovalent ion concentration conductance was lower than 1 nS, suggesting that these peptides form relatively large channels. In contrast to previously published data, selectivity measurements did not show a marked preference between cations and anions in our system. Possible causes of this divergence will be analyzed.

[1] M. L. DeDiego *et al.* (2008) *Virology* 376:379-89.

[2] L. Wilson *et al.* (2004) *Virology* 330:322-31.

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Phosphatidyl-Serine-Positive Cells with High Sensitivity to the Alzheimer's Disease A β Peptides Display Distinctive Mitochondrial Characteristics

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The Alzheimer's A β peptides exhibit cell-selective toxic effects in cultured cells and in neurons of Alzheimer's disease (AD) brains. As we previously showed *ex vivo* neuronal cells and cell lines consisted of subpopulations of cells characterized by differential levels of a common surface membrane target for the phosphatidyl-serine (PtdSer) specific binder annexin V and for the Alzheimer's A β peptides binding. These findings enable us to efficiently separate by flow cytometry sorting PtdSer positive cells on a variety of neuronal cell lines and *ex vivo* neurons and analyze their A β -binding affinity. We collected compelling data confirming the involvement of PtdSer as one of the surface membrane signal molecules for A β . This investigation used the persistent presence of PtdSer on the outer leaflet of the plasma membrane of PC12 cells to identify, sort and further culture the subpopulation of cells that are PtdSer positive (PtdSer⁺) and have high affinity to bind A β , and consequently are more likely to be harmed by A β . Various viability tests showed that the group of cells sorted as PtdSer⁺ was the most sensitive to A β toxic effects. The caspase3/7 activation level of the PtdSer⁺ subpopulation of cells was similar to one of the PtdSer negative (PtdSer⁻) subpopulation and the control cells. Therefore we assume, PtdSer⁺ cells are not undergoing apoptosis. Additionally, PtdSer⁺ cells have persistent higher levels of PtdSer on the outer leaflet of the plasma membrane, significantly lower levels of cytosolic ATP, lower mitochondrial mass and mitochondrial membrane potential, and slightly higher production of reactive oxidative species compared to PtdSer⁻ and control cells. We hypothesize that all of these distinctive cellular and mitochondrial conditions are the basis for the high sensitivity to A β displayed by some selective cell lines and neurons.

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Sublytic Concentrations of Staphylococcus Aureus α -Toxin Trigger Na⁺/K⁺ ATPase Mediated Cell Shrinkage

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Cell lysis can occur through the pathologic action of various membrane-targeting toxins, of which the pore-forming toxin Staphylococcus aureus α -toxin is a prominent example. Paradoxically, S. aureus α -toxin, which forms monovalent cation permeable channels in the host cell plasma membrane, has been observed to cause significant volume shrinkage in many cell types. In HeLa cells we note a \approx 45% decrease in cell volume 30-60 mins post α -toxin treatment in both interphase and mitotic cells. We show that inhibition of the Na⁺/K⁺ ATPase pump with ouabain not only prevents the cell shrinkage, but leads to cell volume expansion after exposure to α -toxin. This suggests α -toxin mediated volume decrease occurs through the upregulation of Na⁺/K⁺ ATPase activity because the 3:2 export:import ratio of Na⁺:K⁺ leads to the loss of one osmolyte per cycle. We therefore conclude that α -toxin induced cell shrinkage is an active cellular process driven by the Na⁺/K⁺ ATPase and elucidate sub-lytic mechanisms of pore-forming toxin assault.

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Evidence for a TH6-TH7 Transmembrane Hairpin in the Diphtheria Toxin T-Domain Open Channel State

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Diphtheria toxin has three domains: a C-terminal receptor-binding (R) domain, a central translocation (T) domain, and an N-terminal catalytic (C) domain. After the toxin binds to the target cell and undergoes endocytosis, it reaches an acidic vesicle compartment. Here the T-domain translocates the C-domain across the endosomal membrane to the cytosol, where it acts as a lethal enzyme. T-domain added to the *cis* side of a planar lipid bilayer forms conducting channels in the presence of a pH gradient (e.g., 5.3 *cis*, 7.2 *trans*). Previous work has identified three transmembrane segments of T-domain in the open channel state, corresponding to TH5, TH8 and TH9 in the crystal structure. Residues near either end of the TH6-TH7 segment are located on the *cis* side of the membrane, based on the polarity of voltage-dependent channel block induced by an attached peptide tag, as well as the effect of streptavidin binding to a biotinylated residue. The TH6-TH7 segment (19 residues) is too short to span the bilayer as an alpha-helical hairpin, but it could as an extended hairpin structure. We have constructed a series of mutant T-domains with a single cysteine residue at positions in TH6-TH7, and probed for an effect of sulfhydryl-specific methanethiosulfonate (MTS) reagents on the channel conductance. Interestingly, at positions in the TH6-TH7 loop, MTS-ET reacts much more quickly from the *trans* side than from the *cis* side. (This is despite the *cis*-positive voltage, which drives the cationic MTS-ET into the channel from the *cis* side.) This suggests that these residues are located near the *trans* side, consistent with a hairpin structure. We are comparing the *cis*-side vs. *trans*-side reaction rates of an uncharged reagent (MTS-glucose) to more systematically determine the transmembrane positions of TH6-TH7 residues.

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Development of the Periss Method to Generate GPCR Ligands/Binders from a Random Peptide Library with a Spider Neurotoxin Scaffold

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The *in vitro* evolution technology has been effectively applied to generate peptides specifically bound to target molecule(s). In conventional methods, however, soluble proteins are mostly the target. We previously succeeded in generating IL6 receptor agonists and antagonists by *in vitro* evolution from a random peptide library with a three-finger type neurotoxin scaffold (Naimuddin *et al.*, 2011). The candidates were initially selected by binding to soluble portion of IL6 receptor, and then confirmed by cell-based assay.

To apply *in vitro* evolution techniques to membrane proteins, however, optimal solubilization/reconstitution is critical and is a major bottleneck in the process. To overcome the problems, we have developed a new technique named PERISS (intra periplasm secretion and selection) method. In this method, the peptides and the target membrane protein are expressed in the periplasm and in the inner membrane of *E. coli*, respectively. Interaction between the peptide and the target, and the following selection are achieved in the periplasmic space. The peptides interacting with the target proteins are collected as binding complexes [peptide-target-*E. coli*]. The selected peptide sequence is encoded in the plasmid in *E. coli*, and the corresponding DNA is amplified by PCR to prepare the second round library.

To evaluate the PERISS method, we have expressed the muscarinic acetylcholine receptor m2 subtype in the inner membrane. The peptide library was constructed based on a neurotoxin GTx1-15 (34 aa) with an ICK motif, which we originally isolated from the spider venom and characterized as a T-type calcium channel blocker (Ono *et al.*, 2011). After six round selections by the PERISS method, selected peptides showed convergence in sequences. One of the peptides showed moderate affinity ($K_d^{app} \sim 300$ nM) and subtype selectivity to m2 receptor.

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Peptide-Gated DEG/ENaC Channels from Hydra Magnipapillata are Highly Permeable for Calcium

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DEG/ENaC ion channels are trimeric proteins, forming a heterogeneous group of sodium-selective ion channels sensitive to the inhibitor amiloride. Most family members known so far are impermeable to calcium. Only ASIC 1a (and possibly MEC-4) conduct calcium to a minor extent. To identify ancient properties of this ion channel family, we have recently cloned five new DEG/ENaC